CLAIMS:

1. A method for characterising DNA, which comprises:

(i) providing a population of DNA fragments, each fragment having cleavably attached thereto a mass label for identifying a feature of that fragment;

(ii) separating the fragments on the basis of their length;

(iii) cleaving each fragment in a mass spectrometer to release its mass label; and

(iv) determining each mass label by mass spectroscopy to relate the feature of each fragment to the length of the fragment.

2. A method according to claim 1, which further comprises:

(a) providing at least one DNA single-stranded template primed with a primer; and

(b) generating the population of DNA fragments from the at least one template, wherein the population comprises at least one series of DNA fragments, the or each series containing all possible lengths of a second strand of DNA complementary to the or each template;

wherein the feature of each fragment determined by each mass label relates to a nucleotide or nucleotide sequence at one end of each fragment, so that each nucleotide is related to a position in the template associated with the mass label so as to deduce the sequence of the or each template.

3. A method according to claim 2, wherein the series of DNA fragments is provided by contacting the template in the presence of DNA polymerase with a mixture of nucleotides sufficient for hybridising to the template for forming a second strand of DNA complementary to the template, wherein the mixture further comprises a set of four probes containing all four nucleotides for hybridising to the template in which the nucleotide of each

probe comprises a modified nucleotide which is capable of polymerising to the second strand of DNA but blocked to prevent further polymerisation thereto and which is cleavably attached to the mass label, which mass label is uniquely resolvable in mass spectrometry for identifying the modified nucleotide, and wherein each fragment is terminated with one of the probes.

4. A method according to claim 2, wherein the at least one template is a plurality of templates and the series of DNA fragments is provided by contacting each template in a separate reaction zone in the presence of DNA polymerase with a mixture of nucleotides sufficient for hybridising to the template for forming a second strand of DNA complementary to the template, wherein the mixture further comprises a set of four probes containing all four nucleotides for hybridising to the template in which the nucleotide of each probe comprises a modified nucleotide which is capable of polymerising to the second strand of DNA but blocked to prevent further polymerisation thereto and which is cleavably attached to the mass label, which mass label is uniquely resolvable in mass spectrometry for identifying the modified nucleotide, wherein each fragment is terminated with one of the probes, and wherein each set of mass labels from each set of four probes associated with each reaction zone is different from the other sets of mass labels; and the fragments are pooled before step (ii).

5. A method according to claim 2, wherein the at least one template is a plurality of templates and the series of DNA fragments is provided by contacting each template in a separate reaction zone in the presence of DNA polymerase with a mixture of nucleotides sufficient for hybridising to the template for forming a second strand of DNA complementary to the template, wherein the mixture further comprises a probe containing only one of the four nucleotides for hybridising to the template, the nucleotide of which probe comprises a modified nucleotide which is capable of polymerising to the second strand of DNA but

blocked to prevent further polymerisation thereto, wherein each fragment is terminated with the probe and wherein either the primer or the modified nucleotide of the probe is cleavably attached to the mass label, which mass label is associated with the reaction zone and uniquely resolvable in mass spectrometry from the mass label in the other reaction zones for identifying the modified nucleotide used in the reaction zone; and the fragments are pooled before step (ii).

6. A method according to claim 2, wherein the at least one template is a plurality of templates and the series of DNA fragments is provided by contacting the plurality of templates in each of four separate reaction zones in the presence of DNA polymerase with a mixture of nucleotides sufficient for hybridising to the template for forming a second strand of DNA complementary to the template, wherein the mixture further comprises a probe containing in each of the reaction zones only one of the four nucleotides for hybridising to the template, the nucleotide of which probe comprises a modified nucleotide which is capable of polymerising to the second strand of DNA but blocked to prevent further polymerisation thereto, wherein each fragment is terminated with the probe and wherein the primer is cleavably attached to the mass label, which mass label is associated with the primer and uniquely resolvable in mass spectrometry from the mass labels associated with the other primers used in the reaction zone; and wherein each nucleotide from its corresponding reaction zone is related to its position in the template.

7. A method according to claim 2, wherein the at least one template is four sets of DNA single-stranded templates, each set comprising an identical plurality of DNA single-stranded templates and the series of DNA fragments is provided by contacting each set in a separate reaction zone in the presence of DNA polymerase with a mixture of nucleotides sufficient for hybridising to the templates for forming a second strand of DNA

complementary thereto, wherein the mixture further comprises a probe containing in each of the reaction zones only one of the four nucleotides for hybridising to the template, the nucleotide of which probe comprises a modified nucleotide which is capable of polymerising to the second strand of DNA but blocked to prevent further polymerisation thereto, wherein each fragment is terminated with the probe and wherein each of the templates of the four sets is primed with a primer to which the mass label is cleavably attached, which mass label which uniquely resolvable in mass spectrometry from the mass labels corresponding to the other templates and which is relatable to its respective template and its respective reaction zone, wherein the fragments are pooled before step (ii), and each nucleotide from its corresponding reaction zone is related to its position in the template.

8. A method according to claim 2, wherein the at least one template is a plurality of templates and the series of DNA fragments is provided by contacting each set of templates in a separate reaction zone in the presence of DNA polymerase with a mixture of nucleotides sufficient for hybridising to the templates for forming a second strand of DNA complementary thereto, wherein the mixture further comprises a set of four probes containing all four nucleotides for hybridising to the template in which the nucleotide of each probe comprises a modified nucleotide which is capable of polymerising to the second strand of DNA but blocked to prevent further polymerisation thereto and which is cleavably attached to the mass label, which mass label is uniquely resolvable in mass spectrometry for identifying the modified nucleotide, wherein each fragment is terminated with one of the probes, and wherein each set of mass labels from each set of four probes associated with each reaction zone is different from the other sets of mass labels and, before step (ii), the fragments are pooled and the pooled fragments are sorted according to a sub-sequence having a common length of 3 to 5 bases adjacent to the primer to form an array of groups of sorted fragments, wherein each group is

spatially separated from the other groups.

9. A method according to claim 2, wherein the series of DNA fragments is provided by

(i) contacting the template in the presence of DNA polymerase with a mixture of nucleotides sufficient for hybridising to the template for forming a second strand of DNA complementary to the template, wherein the mixture further comprises a set of four probes containing all four nucleotides for hybridising to the templates in which the nucleotide of each probe comprises a modified nucleotide which is capable of polymerising to the second strand of DNA but reversibly blocked to prevent further polymerisation thereto, wherein the step of contacting forms a series of templates containing all possible lengths of the second strand of DNA, each second strand terminated with one of the probes;

(ii) removing unpolymerised nucleotides;

(iii) unblocking the modified nucleotides; and

(iv) contacting the series of templates with an array of oligonucleotide probes, wherein each oligonucleotide probe has a nucleotide sequence of common length 2 to 6, all combinations of sequences are present in the array, and wherein each probe is cleavably attached to the mass label, which mass label is uniquely resolvable in mass spectrometry for identifying the nucleotide sequence.

10. A method according to claim 2, wherein the at least one template is a plurality of primed DNA single-stranded templates, each at a unique concentration, and the series of DNA fragments is provided by

(i) contacting the templates in the presence of DNA polymerase with a mixture of nucleotides sufficient for hybridising to the template for forming a second strand of DNA complementary to the templates, wherein the mixture further comprises a set of four probes containing all four nucleotides

for hybridising to the templates in which the nucleotide of each probe comprises a modified nucleotide which is capable of polymerising to the second strand of DNA but reversibly blocked to prevent further polymerisation thereto, wherein the step of contacting forms a series of templates containing all possible lengths of the second strand of DNA, each second strand terminated with one of the probes;

(ii) removing unpolymerised nucleotides;

(iii) unblocking the modified nucleotides; and

(iv) contacting the series of templates with an array of oligonucleotide probes, wherein each oligonucleotide probe has a nucleotide sequence of common length 2 to 6, all combinations of sequences are present in the array, and wherein each probe is cleavably attached to the mass label, which mass label is uniquely resolvable in mass spectrometry for identifying the nucleotide sequence.

11. A method according to claim 2, wherein the series of DNA fragments is provided by contacting the template in the presence of DNA ligase with a mixture of oligonucleotides sufficient for hybridising to the template for forming a second strand of DNA complementary to the template, the oligonucleotides each having a common length in the range 2 to 6, wherein the mixture further comprises a set of probes containing all possible oligonucleotides of the common length 1 for hybridising to the templates in which the oligonucleotide of each probe comprises a modified oligonucleotide which is capable of ligating to the second strand of DNA but blocked to prevent further ligation thereto and which is cleavably attached to the mass label, which mass label is uniquely resolvable in mass spectrometry for identifying the modified oligonucleotide, and the series of fragments contains all possible lengths of the second strand of DNA of integer multiples of 1, in which each fragment is terminated with one of the probes.

12. A method according to claim 2, wherein the at least one template is a plurality of primed DNA single-stranded templates, each at a unique concentration, and the series of DNA fragments is provided by contacting the templates in the presence of DNA ligase with a mixture of oligonucleotides sufficient for hybridising to the templates for forming a second strand of DNA complementary to the templates, the oligonucleotides each having a common length in the range 2 to 6, wherein the mixture further comprises a set of probes containing all possible oligonucleotides of the common length l for hybridising to the templates in which the oligonucleotide of each probe comprises a modified oligonucleotide which is capable of ligating to the second strand of DNA but blocked to prevent further ligation thereto and which is cleavably attached to the mass label, which mass label is uniquely resolvable in mass spectrometry for identifying the modified oligonucleotide, and the series of fragments contains all possible lengths of the second strand of DNA of integer multiples of l, in which each fragment is terminated with one of the probes.

13. A method according to claim 5 ~~or claim 6~~, wherein the plurality of single-stranded templates is primed by hybridising to a known sub-sequence common to each of the templates an array of primers each comprising a base sequence containing a common sequence complementary to the known sub-sequence and a variable sequence of common length, in the range 2 to 6, in which the array contains all possible sequences of that common length and the mass label cleavably attached to each primer is relatable to the variable sequence, which variable sequence is relatable to the particular template to be sequenced.

14. A method according to claim 8, wherein the step of sorting the pooled fragments comprises contacting the fragments with an array of spatially separate oligonucleotides each comprising a base sequence containing a common sequence complementary to the primer sequence of the fragments and a

variable sequence of the common length, which array contains all possible variable sequences of the common length.

15. A method according to ~~any one of claims 4 to 7~~ claim 4, wherein the reaction zones are separate containers.

16. A method according to ~~any one of claims 3 to 10 or 13~~ claim 3 to 15, wherein the mixture of nucleotides comprises ATP, TTP, CTP and GTP.

17. A method according to ~~any one of claims 2 to 10 or 13~~ claim 2 to 16, wherein the modified nucleotides are dideoxy- or deoxynucleotides.

18. A method according to ~~any one of claims 2 to 17~~ claim 2, wherein the primed DNA is immobilised on a solid support.

19. A method according to ~~any one of the preceding claims~~ claim 1, wherein the step of separating the fragments on the basis of their length is effected by capillary electrophoresis.

20. A method according to ~~any one of the preceding claims~~ claim 1, wherein each mass label is cleavably attached to a fragment by a linker cleavable in a mass spectrometer.

21. A method for characterising DNA, which comprises

(a) providing a primed DNA single-stranded template;

(b) contacting the template in the presence of DNA polymerase with a mixture of nucleotides sufficient for hybridising to the template for forming a second strand of DNA complementary to the template, wherein the mixture further comprises a set of four probes containing all four nucleotides for hybridising to the templates in which the nucleotide of each probe comprises a

modified nucleotide which is capable of polymerising to the second strand of DNA but reversibly blocked to prevent further polymerisation thereto, wherein the step of contacting forms a series of templates containing all possible lengths of the second strand of DNA, each second strand terminated with one of the probes;

(c) removing unpolymerised nucleotides;

(d) unblocking the modified nucleotides;

(e) contacting the series of templates with an array of oligonucleotide probes to form a series of fragments, each oligonucleotide probe having a nucleotide sequence of common length 2 to 6, and all combinations of sequences being present in the array, wherein each probe is cleavably attached to a corresponding mass label uniquely resolvable in mass spectrometry for identifying the nucleotide sequence;

(f) separating the fragments from one another on the basis of their length;

(g) cleaving each fragment to release its mass label; and

(h) determining each mass label by mass spectrometry to relate its corresponding nucleotide sequence to a position in the template so as to deduce the sequence of the template.

22. A method for characterising DNA, which comprises

(a) providing a plurality of primed DNA single-stranded templates, each at a unique concentration;

(b) contacting the templates in the presence of DNA polymerase with a mixture of nucleotides sufficient for hybridising to the template for forming a second strand of DNA complementary to the templates, wherein the mixture further comprises a set of four probes containing all four nucleotides for hybridising to the templates in which the nucleotide of each probe comprises a modified nucleotide which is capable of polymerising to the second strand of DNA but reversibly blocked to prevent further polymerisation thereto, wherein the step of contacting forms a series of templates containing all possible

lengths of the second strand of DNA, each second strand terminated with one of the probes;

(c) removing unpolymerised nucleotides;

(d) unblocking the modified nucleotides;

(e) contacting the series of templates with an array of oligonucleotide probes to form a series of fragments, each oligonucleotide probe having a nucleotide sequence of common length 2 to 6, and all combinations of sequences being present in the array, wherein each probe is cleavably attached to a corresponding mass label uniquely resolvable in mass spectrometry for identifying the nucleotide sequence;

(f) separating the fragments from one another on the basis of their length;

(g) cleaving each fragment to release its mass label; and

(h) determining the identity and amount of each mass label by mass spectrometry to relate its corresponding nucleotide sequence to a position in its respective template so as to deduce the sequence of the template.

23. A method for characterising DNA, which comprises

(a) providing a primed DNA single-stranded template;

(b) contacting the template in the presence of DNA ligase with a mixture of oligonucleotides sufficient for hybridising to the template for forming a second strand of DNA complementary to the template, the oligonucleotides each having a common length in the range 2 to 6, wherein the mixture further comprises a set of probes containing all possible oligonucleotides of the common length 1 for hybridising to the templates in which the oligonucleotide of each probe comprises a modified oligonucleotide which is capable of ligating to the second strand of DNA but blocked to prevent further ligation thereto and which is cleavably attached to a corresponding mass label uniquely resolvable in mass spectrometry for identifying the modified oligonucleotide, wherein the step of contacting forms a series of fragments containing all possible lengths of the second strand

of DNA of integer multiples of 1, each fragment terminated with one of the probes;

(c) separating the fragments from one another on the basis of their length;

(d) cleaving each fragment to release its mass label; and

(e) determining each mass label by mass spectrometry to relate its corresponding oligonucleotide to a position in the template so as to deduce the sequence of the template.

24. A method for characterising DNA, which comprises

(a) providing a plurality of primed DNA single-stranded templates, each at a unique concentration;

(b) contacting the templates in the presence of DNA ligase with a mixture of oligonucleotides sufficient for hybridising to the templates for forming a second strand of DNA complementary to the templates, the oligonucleotides each having a common length in the range 2 to 6, wherein the mixture further comprises a set of probes containing all possible oligonucleotides of the common length 1 for hybridising to the templates in which the oligonucleotide of each probe comprises a modified oligonucleotide which is capable of ligating to the second strand of DNA but blocked to prevent further ligation thereto and which is cleavably attached to a corresponding mass label uniquely resolvable in mass spectrometry for identifying the modified oligonucleotide, wherein the step of contacting forms a series of fragments containing all possible lengths of the second strand of DNA of integer multiples of 1, each fragment terminated with one of the probes;

(c) separating the fragments from one another on the basis of their length;

(d) cleaving each fragment to release its mass label; and

(e) determining the identity and amount of each mass label by mass spectrometry to relate its corresponding oligonucleotide to a position in its respective template so as to deduce the sequence of the template.

25. Use of a probe or a set of probes in a method according to ~~any one of claims 1 to 22~~, wherein each probe comprises a modified nucleotide or oligonucleotide which is capable of polymerising to a second strand of DNA complementary to the template but blocked to prevent further polymerisation thereto, which modified nucleotide or oligonucleotide is cleavably attached to a mass label for identifying the modified nucleotide, and wherein each mass label is cleavable from the probe in a mass spectrometer, is resolvable by mass spectrometry and is relatable to its corresponding modified nucleotide or oligonucleotide.

26. Use of a set of oligonucleotide primers in a method according to ~~any one of claims 1, 2, 5, 6, 7 or 13~~, each primer of which comprises a mass label cleavably attached to an oligonucleotide primer base sequence for hybridising to a DNA single-stranded template to form a primed template, wherein each mass label of the set, is cleavable from the primer in a mass spectrometer, is uniquely resolvable in relation to every other mass label in the set by mass spectrometry and is relatable to the oligonucleotide primer base sequence.